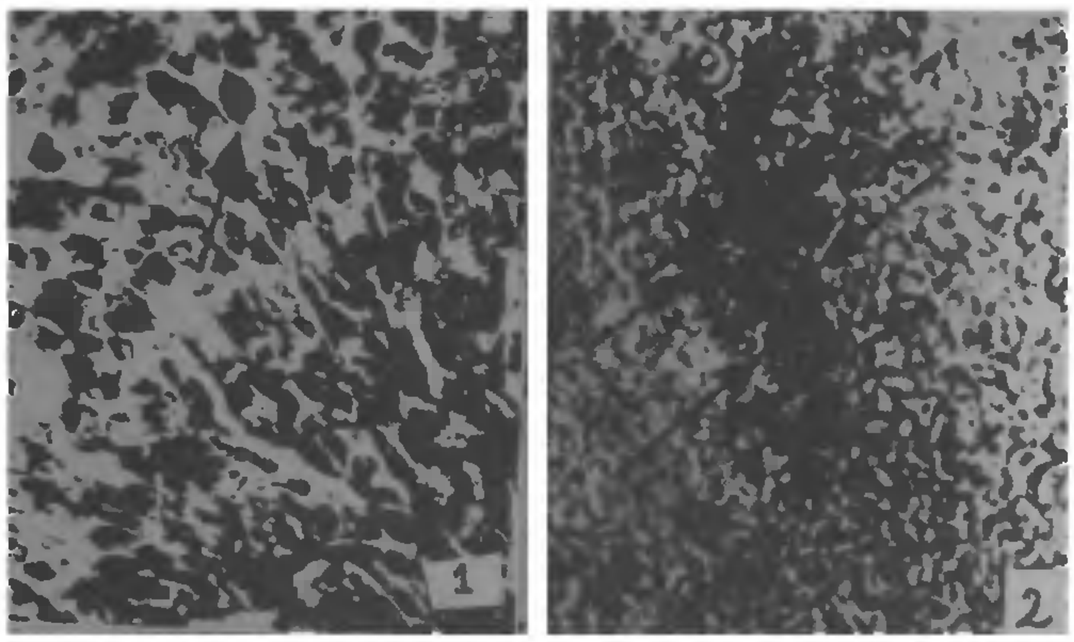

Figures 1, 2. 1. The dark theca luteal cells and faint granulosa cells. **2.** Arrows show degenerating parts of corpus luteum.

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EFFECTS OF BLOCKING OVULATION WITH PENTOBARBITONE ON OVARIAN PROTEINASES IN RATS

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THE detailed mechanism of rupture of the follicle wall in mammalian ovulation is not well known. The mature ovarian follicles contain proteolytic enzymes[1–7], which are one of the many factors involved in the rupture of follicular wall to enable the ovum to escape from it during ovulation [4,7] and the latter is induced by endogenous luteinizing hormone (LH) surge from the pituitary which is under the control of central nervous system[10]. The administration of pentobarbitone sodium (PBS) on the afternoon of proestrous blocks the pre-ovulatory surge of LH and thus ovulation is prevented[8–15]. The biochemical changes induced by PBS injection in previous studies include a decrease in follicular oestradiol production between proestrous and one day afterwards[9–15]. There is no previous report on the effects of PBS on ovarian proteolytic enzymes. Therefore, it was planned to find out the activity of ovarian proteolytic enzymes on different days of the estrous cycle as well as after blockage of ovulation by PBS injection given in the early afternoon of proestrous.

Thirty adult female albino rats, aged 2–3 months, and weighing 150–200 g, provided with pelleted food (Hindustan Levers Ltd) and water *ad libitum* were used. Vaginal smears were examined daily and only the rats showing two or more regular cycles of 4 days length were used. Five animals were sacrificed in each of the stages and the remaining ten were injected with PBS dissolved in normal (0.9%) saline in a dose of 3 mg per 100 g body weight between 14–30 and 15.00 hr in the afternoon of proestrous. The occurrence (in the normal) and blockage (in PBS treated) of ovulation was established by checking the oviducts for the presence of eggs and ovaries for the postovulatory follicles on the morning of estrous.

Preparation of homogenate: The animals were sacrificed by decapitation and the ovaries dissected out in the cold normal saline were cleared of the fat and adhering tissues, weighed, minced and homogenized with 1 ml of 0.01 M $CaCl_2$ containing 0.025% Triton X-100. The homogenate was centrifuged at 4°C for 20 min and diluted to 3 ml total volume, per two ovaries of each animal. The preparations were stored at –20°C and used within one month for enzyme assay.

Enzyme assay: The activity of neutral proteases was measured using 3% casein as a substrate at pH 7.5. The assay media were incubated at 37°C for 1 hr. The reaction was stopped by adding 10% (W/V) TCA. After keeping the solutions at room temperature for at least 15 min, they were centrifuged at 2500 RPM for 10 min. The supernatant was transferred to a clean tube and the absorbance of the supernatant was measured at 280 nm.

The enzyme activity is expressed as the tyrosine

liberated in μg per mg of ovarian tissue. A standard tyrosine curve was made for comparison. The data is expressed as mean ± S.E. and the significance of effect of PBS is analyzed statistically using student's *t* test by taking estrous as the control.

The proteolytic enzyme activity of the rat ovary on different days of the estrous cycle and after PBS treatment is shown in table 1. The enzyme activity was maximum during estrus, decreased on metestrous and again started increasing from diestrous to proestrous for reaching a maximum level during estrous. The increasing order of activity during the cycle was found to be E > P > D > M which shows a specific pattern of change related to ovulation. So far no such information is available on the changes in the level of proteases during the estrous cycle of the rat, although Reichert[18] reported the presence of neutral proteinases in the rat ovary. Tojo *et al*[19] also observed a significant increase in the neutral protease activity in the postovulatory follicle of the domestic hen. In contrast Parr[20] could not detect neutral proteinase activity in rat ovarian follicle walls near the time of ovulation. The discrepancy may perhaps be due to differences in assay conditions.

In the PBS-treated animals, the proteolytic enzyme activity drastically decreased (table 1). It was significantly different from the activity found in the estrous females ($P < 0.01$) indicating the inhibitory effect of PBS on the ovarian proteases involved in the process of ovulation. The inhibitory effect of PBS on ovarian proteolytic enzyme activity between proestrous and one day afterwards correlates with its similar effects on estradiol production[9–15] and it seems that the absence of LH surge caused by PBS injection decreases both follicular estradiol as well as ovarian neutral proteinases. With regard to the possible mechanism whereby the absence of a preovulatory LH surge caused by PBS results in the decreased ovarian proteolytic enzyme activity, it needs to be investigated whether it is due to a direct effect of PBS or PBS-induced decrease in LH secretion or PBS-induced decrease in estradiol production as ovarian steroids are already known to variably affect the ovarian proteases[16, 17] and stimulatory effects of LHRH on plasminogen activators (serine proteases)[21] secreted by granulosa cells in culture have already been suggested.

Table 1 *Ovarian proteolytic enzyme activity during estrous cycle and after pentobarbitone sodium treatment*

No. of rats	State of the estrous cycle	Enzyme activity (tyrosine liberated in μg/mg tissue) mean ±S.E.
5	Estrous	2.05±0.25*
5	Metestrous	0.74±0.02
5	Diestrous	1.26±0.13
5	Proestrous	1.35±0.21
10	Pentobarbitone sodium treated	0.81±0.23*

* The values are significantly different from each other ($P < 0.01$)

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STUDIES ON SPERMATOCYTIC CHROMOSOMES OF AN AQUATIC WOLF SPIDER, *HIPPASA MADHUAE* TIKADER AND MALHOTRA (LYCOSIDAE: ARANEAE)

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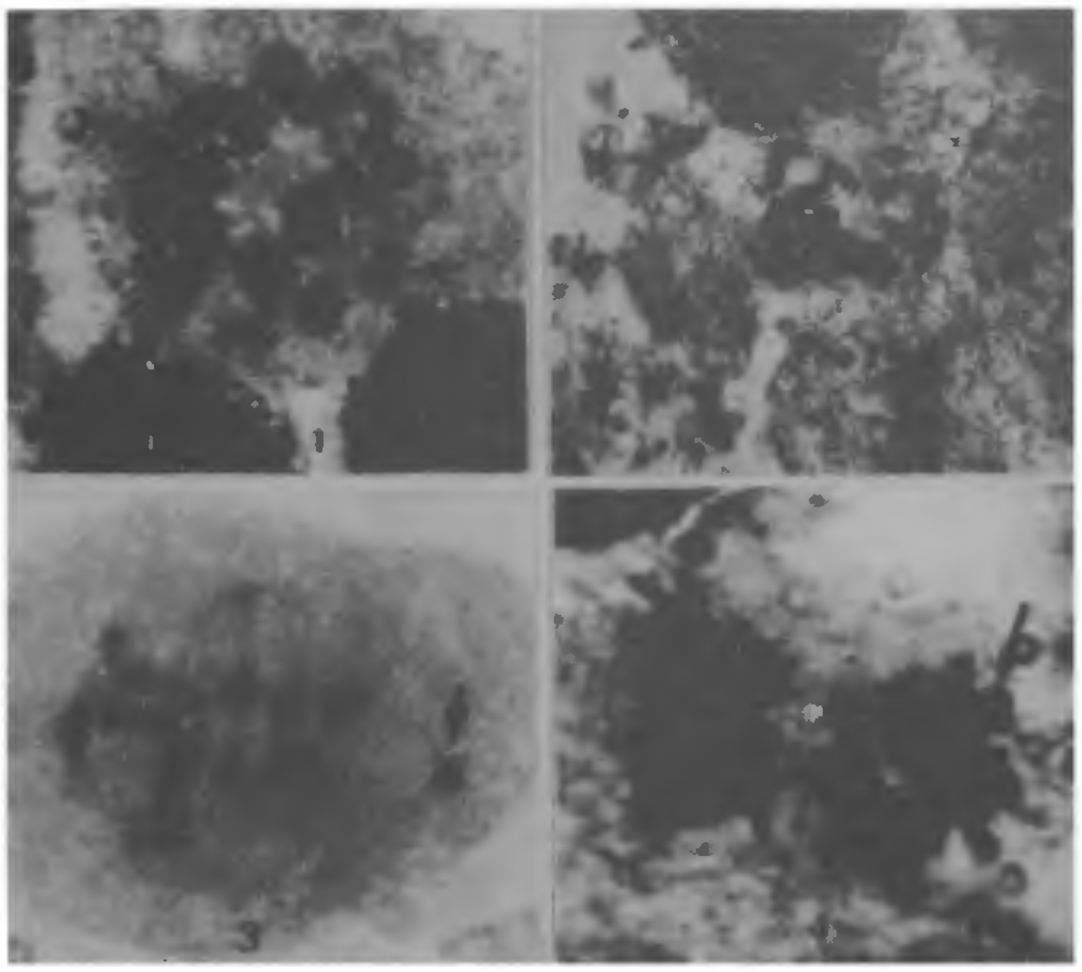
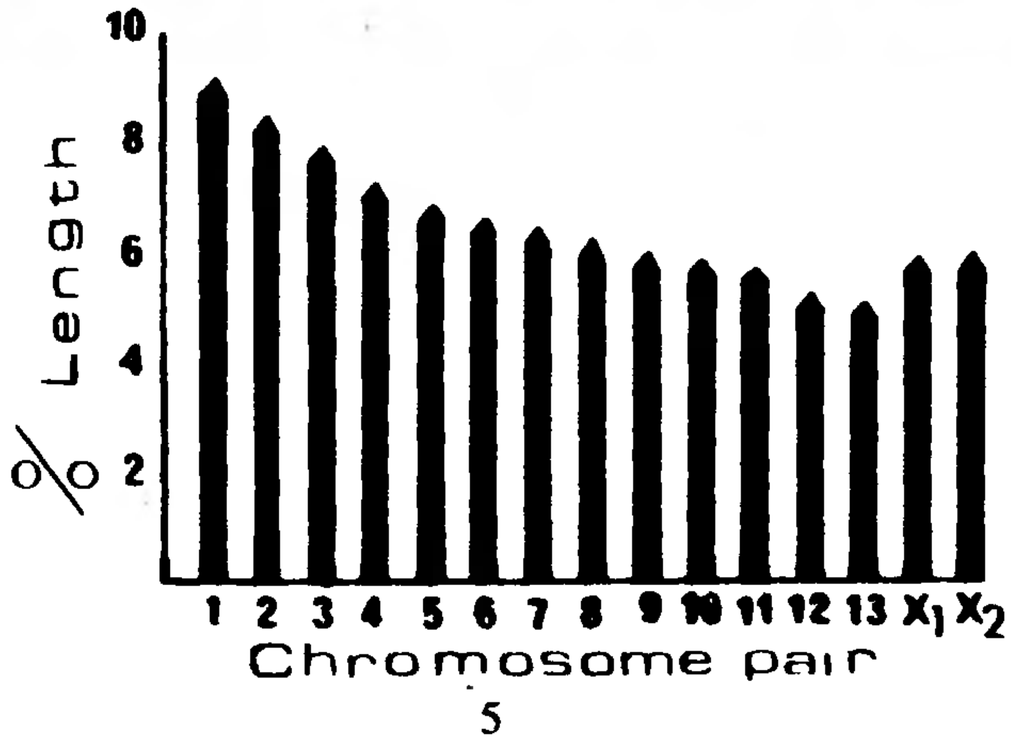
CONSIDERABLE work has been done on terrestrial spiders[1–7]. However, data on the chromosomal survey of the aquatic wolf spider belonging to the family Lycosidae (order Araneae of Arachnida) are quite scanty. The present study reports the behaviour of spermatocytic chromosomes of *Hippasa madhuae*. Sub-adult male spiders (15) collected from weeds grown in the canals of Bhubaneswar, Orissa (58° 53′ E longitude and 20° 21′ N latitude) constitute the material used for the investigation. Permanent chromosome preparations were made from squashed testes following Smith's technique[8].

The diploid chromosome number as determined from spermatogonial metaphase plate is 28. All the chromosomes are acrocentric and the bivalents are scattered throughout the nuclear area (figures 1 and 2). The metrical analyses of spermatogonial chromosomes scored from ten well-spread metaphase plates were made (table 1) and the idogram (figure 5) was prepared on the basis of percentage length of each chromosome as calculated upon the total chromosome length (TCL). During early spermatocytic prophase two dark stained condensed chromatin structures representing the sex chromosomes are discernible. The positive heteropycnotic behaviour of the chromosomes is exhibited up to the diplotene stage. Out of 28 chromosomes, 26 are autosomes and two are sex chromosomes (X_1 and X_2). The multiple sex chromosomes lie very close to each other and from an accessory plate (figure 3). Chromosomes of anaphase I are however, comparatively larger than at metaphase (figure 4). During this stage the autosomes and sex chromosomes





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Figures 1–5. **1.** Late diakinesis. **2.** Metaphase I. **3.** Metaphase I with accesory plate showing sex chromosome complex. **4.** Anaphase I. **5.** Idiogram.

Table 1 *Mean length and percentage of haploid set of chromosomes of aquatic spider Hippasa madhuae*

Pair No.	Mean Length ± S.E. (μ)	Percentage
1	3.28 ± 0.04	9.18
2	3.04 ± 0.08	8.51
3	2.84 ± 0.02	7.95
4	2.66 ± 0.09	7.45
5	2.52 ± 0.10	7.05
6	2.42 ± 0.06	6.77
7	2.32 ± 0.07	6.49
8	2.27 ± 0.06	6.35
9	2.18 ± 0.05	6.10
10	2.11 ± 0.04	5.91
11	2.10 ± 0.03	5.88
12	1.85 ± 0.18	5.18
13	1.83 ± 0.18	5.12
X_1 (unpaired)	2.14 ± 0.03	5.99
X_2 (unpaired)	2.14 ± 0.03	5.99